

TEXAS MEDICAL CENTER NASA/JOHNSON SPACE CENTER

COOPERATIVE AGREEMENT PROGRAM NCC 9-36, ROUND II

COVER SHEET FOR FINAL REPORT

1N-51
026063

Name of Subcontractor: JoAnn Trial, Ph.D.

Title:

Institution: Baylor College of Medicine

Name of Project: The Study of Leukocyte Functions in a Rotating
Wall Vessel

Amount of Grant: ~~400,000~~

* Amount Spent, if Different from Amount Granted:

Date Project Was Completed:

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1. Description of research project.

The objective of this study was to investigate the behavior of leukocytes under free-fall conditions in a rotating wall vessel. In such a vessel, the tendency of a cell to fall in response to gravity is opposed by the rotation of the vessel and the culture medium within, keeping the cells in suspension without fluid shear. Previous reports indicated that such functions as lymphocyte migration through collagen matrix or monocyte cytokine secretion are altered under these conditions, and these changes correlate with similar functional defects of cultured cells seen during spaceflight.

2. Achievements and outcomes.

Because one of the most critical functions of leukocytes as the first line of defense against invading organisms is phagocytosis and the associated oxidative burst, these functions were investigated in this model. Phagocytes were cultured in tubes or in a vessel rotating around a horizontal axis, in which the cells and added microorganisms remained in free fall. Other samples were maintained in a control vessel that was either stationary or rotating around a vertical axis. Cells and organisms in the latter vessel fell to its floor just as in a tube at one gravity, but with the added factors of gas exchange and vibration specific to vessel culture. Cultures were performed by diluting heparinized normal donor human blood 1:4 with culture medium. At this dilution, microorganisms were still efficiently opsonized by the antibodies and complement present in the blood, the blood was still anticoagulated, and the cells were not activated as they might be by isolation procedures. The diluted blood was cultured for various periods of time before microorganisms were added to the cultures. The microorganisms were labeled with the fluorescent dye, Texas Red, so that cells taking up the organisms could be detected by flow cytometry. Samples were removed from the cultures ninety minutes after the addition of the organisms and the harvested cells were stained with phycoerythrin-conjugated anti-CD14 to distinguish monocytes from neutrophils. To assess the number of cells that could undergo an oxidative burst associated with the phagocytosis, the cells were exposed to dichlorodihydrofluorescein diacetate before the culture. This colorless dye enters cells, where it is retained after cleavage by esterases. Oxidation of the molecule by hydrogen peroxide during an oxidative burst generates the fluorescent dye, fluorescein, which can be measured by cytometry. For analysis, neutrophils were identified by their light scatter characteristics, and then assessed for their red fluorescence indicating phagocytosis of organisms, and their green fluorescence indicating the presence of an oxidative burst. Monocytes were identified both by their light scatter characteristics and their orange phycoerythrin fluorescence before being analyzed for

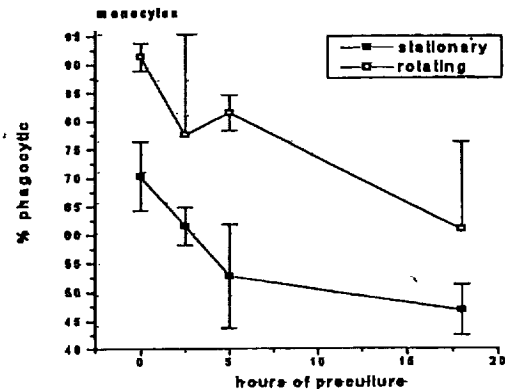
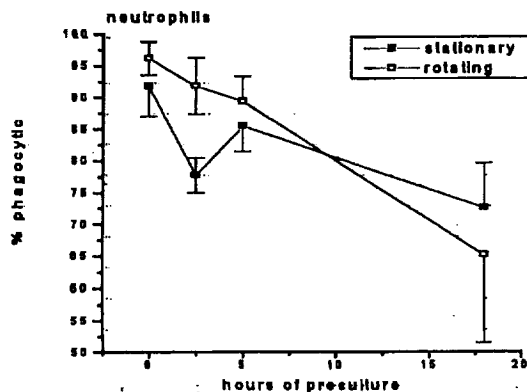


Figure 1. Diluted blood was cultured in stationary tubes or in rotating vessels for the indicated time before the addition of *Staphylococcus aureus* labeled with Texas Red at a 10:1 ratio to leukocytes for ninety minutes. Harvested cells were stained for CD14 and red cells lysed before enumeration by flow cytometry. Data indicate means and standard errors of four normal human donors.

phagocytosis and an oxidative burst as for neutrophils. In Figure 1, the number of neutrophils (left panel) and monocytes (right panel) able to undergo phagocytosis after various culture periods before the addition of organisms indicates that there is no defect in phagocytic function when the cells are in free fall for up to eighteen hours. The enhancement of phagocytosis in the rotating vessel as opposed to stationary cultures may reflect a somewhat greater collision frequency of cells and organisms during rotation.

When phagocyte responses to other organisms were assessed, similar results were seen. The gram positive bacterium *Staphylococcus aureus*, the gram negative bacterium *Escherichia coli*, and the fungal organism *Candida albicans* were given to leukocytes after four hours of culture and the levels of phagocytosis and oxidative burst measured. In these experiments the function measured at time zero (gray bars) was compared to that in a stationary vessel (empty bar) or in a rotating vessel (hatched bar) as shown in Figure 2. The phagocytic function of neutrophils (upper left panel) and monocytes (upper right panel) did not differ under stationary versus rotating conditions, and neither did the oxidative burst of neutrophils or monocytes (data not shown).

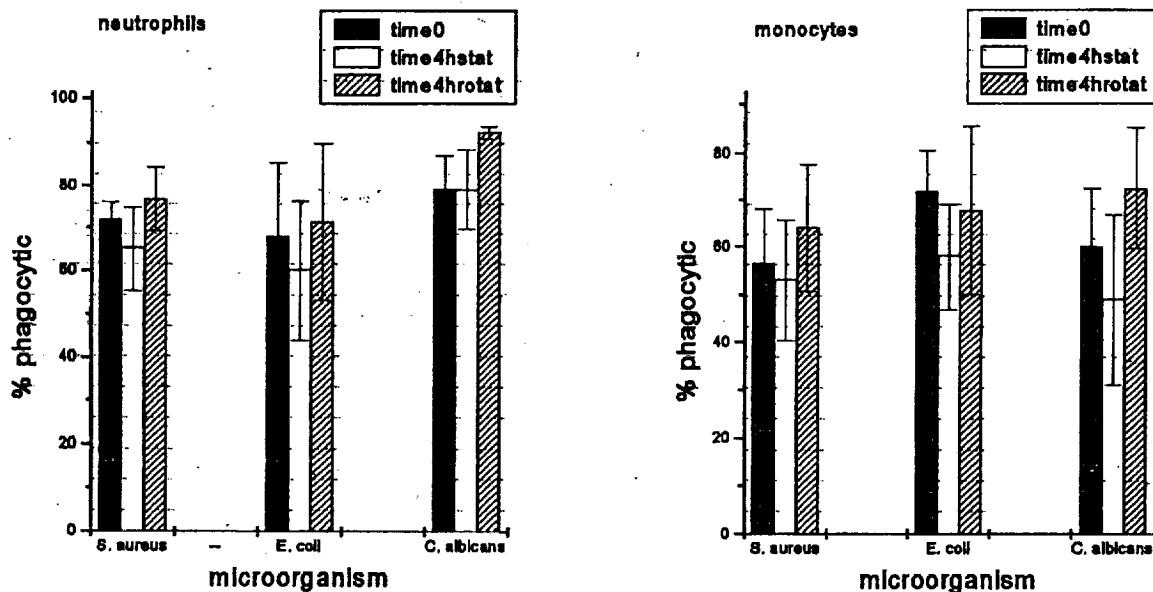


Figure 2. Diluted blood was assessed for phagocytosis immediately after being drawn (time 0) or after four hours of culture in rotating or stationary vessels. *S. aureus* was added at a 10:1 ratio to leukocytes, *E. coli* at 50:1, and *C. albicans* at 4:1 for ninety minutes. Data are means and standard errors of six donors.

Another function of leukocytes necessary for defense against invading microorganisms is the ability to migrate through the endothelium lining blood vessel walls into tissues. Transendothelial migration has been modeled in vitro by growing a

confluent monolayer of human endothelial cells on a hydrated collagen pad. Mononuclear leukocytes (lymphocytes and monocytes) migrating through the endothelium are trapped in the collagen pad and can be harvested by collagenase digestion. The freed leukocytes are stained with anti-CD14 to distinguish monocytes, and are counted by flow cytometry. To adapt this in vitro model to one that would allow the system to remain in free fall, the endothelial cells were grown on collagen-coated beads (Cytodex-3) small enough to remain in suspension in the rotating vessel. When the endothelial cells became confluent over the entire surface of the bead, leukocytes labeled with a fluorescent supravital dye were added to the bead suspension in the vessel. These cells migrated through the endothelial monolayer into the bead.

The migrated leukocytes were harvested by collagenase digestion of the beads, and the collected cells stained with an antibody against CD14 to distinguish monocytes from lymphocytes. The number of migrated cells were then counted by flow cytometry. In Figure 3 is shown the number of lymphocytes (left panel) and monocytes (right panel)

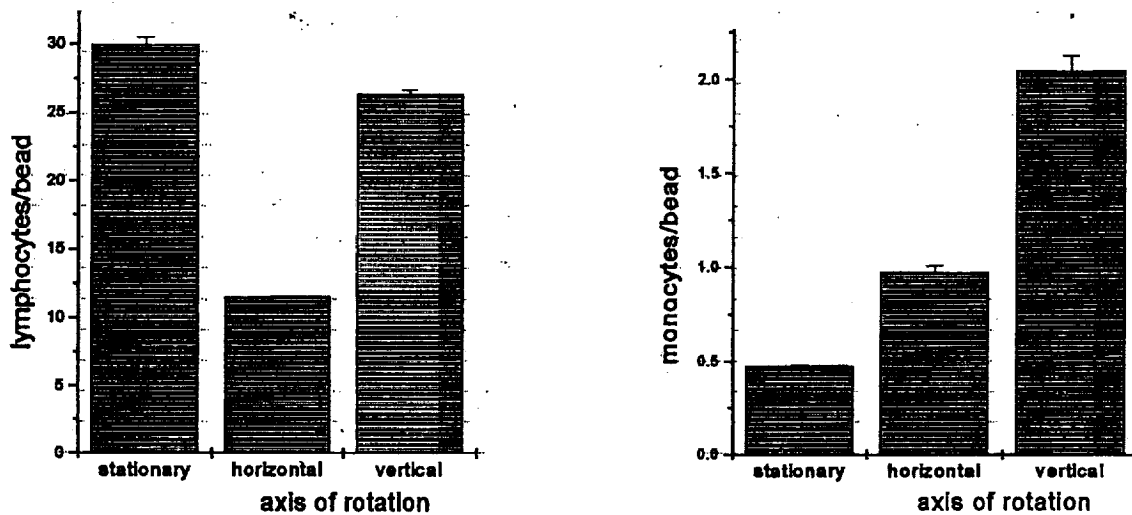


Figure 3. Number of lymphocytes and monocytes migrated through endothelium covering Cytodex-3 beads cultured in a stationary vessel, or vessels rotated with horizontal or vertical axes. Data are means and standard errors of three individual donors.

that migrated per bead. The numbers of lymphocytes did not differ between stationary vessels and those rotating around a vertical axis (in which cells and beads fell to the floor). However, in vessels turning around a horizontal axis, in which cells and beads remained in suspension, there was a significant defect in the ability of lymphocytes to migrate through endothelium. This could not be due to a failure of the cells to collide with the beads, because monocytes in the cultures showed an increased rate of migration through the same beads (right panel). To investigate the mechanisms responsible for the lymphocyte defect, cultures of leukocytes from six individuals were studied for changes in adhesion molecule expression under the same three conditions. No consistent changes due to vessel rotation were seen.

In summary, some leukocyte functions are affected by free fall, whereas others are not. Monocyte and neutrophil functions such as phagocytosis, oxidative burst, and

transendothelial migration are marginally enhanced, and are certainly not defective. Lymphocyte transendothelial migration was compromised by free-fall conditions, but not totally suppressed. Understanding the mechanism of this defect may relate to the observed suppression of delayed-type hypersensitivity seen in vivo during spaceflight, since this phenomenon depends on the transendothelial migration of lymphocytes.